The Occurrence and Control of Pepper Mild Mottle Virus (PMMoV) in the USDA/ARS *Capsicum* Germplasm Collection

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ABSRACT

Four thousand-four-hundred and three seed inventories of *Capsicum* spp. obtained from the USDA/ARS *Capsicum* germplasm collection were tested for the presence of Pepper mild mottle virus (PMMoV). Approximately 32% of these inventories tested positive for PMMoV and the virus distribution was nearly uniform across the five cultivated species of this genus. Treatment of PMMoV-infected *Capsicum* seed with 10% trisodium phosphate (TSP) for 2.5 h at room temperature reduced germination in 11/50 accessions examined and significantly increased the number of abnormal seedlings. Indicator plant [*Chenopodium giganteum* D. Don.—formerly *C. amaranticolor* (Coste & A. Reyn.) Coste & Reyn] analysis revealed that treatment of PMMoV-infected *Capsicum* seed with 10% TSP for 2.5 h reduced virus infectivity, but did not eliminate it. Treatment with 10% TSP for 24 h eliminated PMMoV from four of six tested accessions. Short-term treatment of PMMoV-infected *Capsicum* seed with TSP prior to planting is expected to significantly reduce the incidence of the virus, but not eliminate it in all instances.

INTRODUCTION

Pepper mild mottle virus (PMMoV) is one of the major viral pathogens in *Capsicum* spp. (Genda et al., 2005) and frequently results in significant crop losses in field and greenhouse plantings. The virus occurs in North America (Adkins et al., 2001; Beczner et al., 1997), Australia (Pares 1985), Japan (Honda and Kameya-Iwaki, 1991; Ikegashira et al., 2004), China (Xiang et al., 1994; Wang et al. 2006), Taiwan (Green and Wu, 1991), Europe (Wetter et al., 1984; Alonso et al., 1989; Marte and Wetter, 1986) and North Africa (Mnari-Hattab and Ezzaier, 2006). An outbreak of the disease occurred in the southeastern US and caused significant losses to plantings of jalapeno peppers (Martinez-Ochoa et al., 2003). PMMoV has also caused recent and significant economic losses in Japanese (Hagiwara et al., 2002) and Chinese (Wang et al., 2006) field and greenhouse pepper production areas. The virus is now assumed to be distributed worldwide (Lamb et al. 2001).

Symptoms that result in response to plant infection with PMMoV may include puckered and mottled yellow or light green leaves, and plants may be stunted. However, foliar symptoms are frequently mild which can thwart efforts to identify and rogue infected plants at an early stage of development. Fruit size is typically reduced and older fruit may be distorted in shape with streaks or necrotic lesions (Brunt et al., 1996). PMMoV is a member of the

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genus *Tobamovirus* (Togaviridae). It is a positive-sense single-stranded RNA virus with a ~6,400 base pair monopartite genome that encodes at least four proteins including: 130 K and 180 K replication proteins, a movement protein, and a coat protein (CP). The genome is encapsidated in a rod-shaped particle (Wetter et al., 1984). The virus genome is considered highly stable with a single prevailing haplotype from which closely related variants arise (Rodriguez-Cerezo et al., 1989). PMMoV was shown by Wetter et al. (1984) to systemically infect cultivated *Capsicum annuum* L, *C. frutescens* L. and *C. chinense* Jacq. Its host range is similar, but not identical to, other tobamoviruses (Wetter et al., 1984). Various attenuated strains of PMMoV have been developed, though none has provided significant levels of cross-protection (Tsuda et al., 2007).

As is common among tobamoviruses (Ikegashira et al., 2004) PMMoV is transmitted through the seed and infects the emerging radicle as it comes into contact with the infected seed coat, or subsequently through wounds on plant roots, stems or leaves as a result of abrasions during (Demski, 1981) or after transplanting. The virus can retain its infectivity when seed have been stored for prolonged periods (Genda et al., 2005). Control of the disease in the field is difficult. Diseased plant material remains infectious for long periods of time in the soil (Ikegashira et al., 2004) and can readily re-infect subsequent crops (Lamb et al., 2001). The virus is spread as a result of common field practices including pruning and harvesting. The use of stringent phyosanitary procedures has been recommended as a means of control (Beczner et al., 1997). The most effective means to control PMMoV is avoidance (Lamb et al., 2001). Wang et al. (2006) and others (Lamb et al., 2001) have emphasized the need for certified seed and the development of an effective seed treatment. However, no chemical control measures other than methyl bromide (Yoneyama, 1998) have proven effective for disease control in the field or greenhouse. Treatment of infected seed with 10% trisodium phosphate (TSP) has, however, been recommended as a means to reduce the levels of active virus in seed (Lamb et al., 2001; AVRDC, 2004).

The *Capsicum* spp. germplasm collection in the USDA/ARS Plant Genetic Resources Unit (PGRU) in Griffin, GA currently maintains and distributes seed of more than 5,000 accessions of *Capsicum* spp. (Jarret et al., 1990). The collection contains the five cultivated species (*C. annuum*, *C. frutescens*, *C. baccatum*, *C. chinense* and *C. pubescens*), non-cultivated *Capsicum* species, and species of related genera. Seed of all materials are made available to scientists and educators worldwide in support of plant science research and educational and agri-business activities. In 2005, the Griffin genebank was notified that several accessions received from the genebank were infected with PMMoV. This study was undertaken to examine the extent of the infection of the genebank's materials with this virus, and to evaluate the efficacy of TSP treatment as a potential means to eliminate it from selected seed lots.

MATERIALS AND METHODS

The PGRU *Capsicum* collection contains multiple inventories of most accessions maintained there. Seed or plant material collected and/or donated directly to the genebank is classified as original seed. As seed inventories are reduced,

fresh seed are produced. These subsequent inventories are referred to as first regeneration, second regeneration, etc. Unless noted otherwise, all seed lots referred to in this study were first regeneration.

ELISA screening for PMMoV

Two hundred and fifty seed (or 10% of the available seed inventory — whichever was less) of each of 4403 accessions of *Capsicum* spp. in the S-9 genebank (Jarret et al., 1990) were sent to Agdia, Inc. (Elkhart, IN) where lots were individually tested for the presence of PMMoV using direct-antigen-coating enzyme-linked immunosorbent assay (DAC-ELISA). In some instances, samples of 50 seed or leaf tissues were tested for PMMoV in Griffin using antiserum obtained from Agdia and the ELISA protocols described by Gillaspie et al. (1995). Absorbance at 405 nm was measured using an Emax Microplate Reader (Molecular Devices Corp., Sunnyvale, CA).

TSP treatment of seed

Seed to be treated were placed in 5 × 8 cm nylon mesh bags and completely immersed in 10% (w/v) trisodium phosphate ($Na_3PO_4 \cdot 12H_2O$, Fisher Scientific, St. Louis, MO) at room temperature (25 ± 1 °C) for the allotted time with continuous agitation. Seed were subsequently rinsed under running tap water for 2 h (changed at 20 min intervals) and dried at 20 °C (25 to 30% relative humidity) for one week. Control seed were either not treated or immersed in ddH_2O for an identical time and dried in a similar manner.

Germination testing

Germination testing was conducted according to the rules established by the Association of Official Seed Analysts (AOSA, 2005). One hundred seed per accession (replicated three times) were placed on paper towels moistened with ddH_2O containing 0.2% (w/v) KNO_3 and incubated at 20 °C for 12 h (dark) and 30 °C for 12 h (8 h light). Data on germination were recorded after days six and 14. Normal and abnormal (damaged or deformed) seedlings were counted and recorded separately, as were dead and decayed seed.

Indicator plant assays for and maintenance of PMMoV

Samples of various pepper lines believed to be infected with the virus based on the results obtained previously from Agdia were ground in 0.25 M phosphate buffer (pH 7.4) (Gillaspie et al., 1995) and these were tested by DAC-ELISA. All seed lots tested positive. Additional seed (50) from these lots were then treated with 10% TSP for various times and subsequently rinsed and dried for storage. Treated and control seed samples were ground in the phosphate buffer noted previously with a mortar and pestle. The extract from each sample was mechanically inoculated onto healthy 6 to 8 week old *Chenopodium giganteum* plants in a greenhouse. Symptoms were recorded two weeks after inoculation. Infected plants were extracted in buffer and mechanically inoculated onto lines of healthy peppers (PI 441606 and Jalapeno M). Plants were subsequently grown in a growth chamber at 28 °C (12 h photoperiod)

and tested by DAC-ELISA. In a follow-up test, seed of six accessions (PI nos. 222133, 439216, 439258, 439485, 508433 and 596052) that were heavily infected with PMMoV based on earlier ELISA results were soaked in 10% TSP for 24 h, rinsed and dried. These seed samples were then ground in phosphate buffer and used to inoculate *C. giganteum* (five plants/treatment). Virus symptoms were evaluated two weeks after inoculation.

Sequencing of the PMMoV coat protein

The identity of the virus was also confirmed by RT-PCR. Total RNA extracts of infected pepper leaves were isolated using the RNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA). A set of primers was designed using Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) with PMMoV CP sequence data obtained from Genebank. The forward primer was 5'-TACTTCGGCGT TAGGCAATC-3' and the reverse was 5'-GGAGTTGTAGCCCAGGTGAG-3'. For first strand c-DNA synthesis, an RT mix (20 µl, consisting of 4 µl of 5X first strand RT buffer [Invitrogen, Carlsbad, CA], 2 µl each of 0.1 M dithiothreitol and 10 mM deoxynucleotide triphosphate [dNTP], 0.25 µl of SUPERSCRIPT RT RNase H^- Reverse Transcriptase [Invitrogen], 0.2 µl RNasin RNase inhibitor [Promega Corp., Madison, WI], 8.5 µl nuclease-free water, and 0.5 µl of the reverse primer [primers at 100 pmol/µl]), was added to 3 µl of total RNA from test tissue. The reaction mix was then incubated at 37 °C for 1 h followed by treatment at 94 °C for 2 min to inactivate the enzyme.

Viral cDNA was amplified in 25 µl PCR reactions containing 2.5 µl of 10X PCR buffer (Promega Corp., Madison, WI), 3.5 µl of 25 mM $MgCl_2$, 2.5 µl of 2 mM dNTP, 0.2 µl (25 pmol) each of forward and reverse primers, 0.2 µl (1 U) of *Taq* DNA polymerase (Promega), 13.9 µl of nuclease-free water, and 2 µl of RT product as follows: 94 °C, 2 min; 35 cycles of 94 °C, 30 sec; 54 °C, 30 sec; 72 °C, 60 sec; and one cycle of 72 °C, 10 min. PCR amplification was assessed by electrophoresis in a 1.5% agarose gel in TBE buffer (89 mM Tris-borate and 2 mM EDTA, pH 8.3) and stained in ethidium bromide. The expected product size for PMMoV was ~387 bp.

The RT-PCR product was isolated by excising the band from an agarose gel and centrifuging the extract through a Wizard minicolumn (Promega). The amplicon (20 ng) was then subjected to PCR amplification with the above protocol. Sequencing reactions were prepared using a DTCS quick start sequencing kit (Beckman Coulter, Fullerton, CA). The sample was sequenced bidirectionally on a Beckman CEQ 8000 using the LFR-1 method. The plasmid PUC18 was also sequenced as a positive control. The sequence module of the software package CEQ 8000 Genetic Analysis System version 8.0.52 (Beckman Coulter) was used to call the bases. The forward and reverse strands were aligned using AlignIR version 2.0 (LI-COR, Lincoln, NE). This process was repeated with similar results. These results were then compared to PMMoV and other viral sequences via a BLAST search.

Data analysis

Statistical analysis of germination data was performed using SigmaStat 3.1.

RESULTS AND DISCUSSION

A total of 4403 inventories of *Capsicum* spp. seed were screened for the presence of PMMoV. The seed of these first regeneration inventories were derived from plants initiated from seed that were received by the genebank over a period of more than 60 years and subsequently regenerated in a variety of locations over a similar period. The indexing revealed widespread distribution of PMMoV [1397 (31.7%) infected inventories] within the collection (Table 1). No obvious differences in the percentages of infected inventories were observed among the various species, except *C. annuum* var. *glabriusculum* — a wild (but edible-fruited) species often referred to as chiltepin (DeWitt and Bosland, 1996). However, this may be due to the small number of seed lots tested.

To evaluate the presence of the virus in the seed as initially collected or donated to the genebank, an additional 150 inventories of original seed were indexed for PMMoV. Fifty-five (~36.6%) of these original seed lots tested positive for PMMoV. This was similar to the average of the previous test results (~32% positive) utilizing first regeneration seed. Seventy-one percent of the first regeneration inventories derived from original seed that had tested positive for PMMoV also tested positive for PMMoV, indicating that the virus was not always transmitted from seed to plant, but that it was transmitted at a relatively high rate and/or that the virus had not been transmitted from seed to plant but had been reacquired in the field or greenhouse during regeneration. Approximately 50% of the first regeneration inventories that were derived from original seed lots that had tested negative for PMMoV tested positive for PMMoV, indicating that high numbers of accessions had become infected during regeneration.

TABLE 1. Distribution of PMMoV among *Capsicum* seed lots in the USDA/ARS germplasm collection.

Species	No. tested	No. infected	% infected
C. annuum var. *annuum* L.	3046	929	30.4
C. annuum var. *glabriusculum* (Dunal) Heiser & Pickersgill	39	39	100
C. baccatum L.	394	148	37.5
C. chacoense Hunz.	24	8	33.3
C. chinense Jacq.	448	155	34.6
C. flexuosum Sendtn.	3	3	100
C. frutescens L.	243	81	18.6
C. galapagoense Hunz.	1	0	0
C. pubescens Ruiz & Pav.	59	11	18.6
Unidentified *Capsicum* spp.	182	70	38.5
Tubocapsicum anomalum (Franch. & Sav.) Makino	1	1	100
Tubocapsicum spp.	1	1	100

To confirm the identification of the virus using nucleotide sequence similarity, ~387 bp of the CP gene of PMMoV was isolated and sequenced from PI 439485. A GenBank BLAST search indicated that the ~387 bp of the CP fragment from PI 439485 was identical to those isolated from PMMoV strains from Brazil and Japan as reported by Eiras et al. (2004) and Hamada et al. (2007), respectively. Since seed lots infected with PMMoV (as determined by ELISA) were originally acquired from different countries over a long period of time, it is reasonable to assume that more than a single strain of PMMoV is present, and that one or more of these have not been previously described.

Alexander (1960) reported that treatment of tomato seed with TSP or HCl was effective in reducing, but not eliminating, TMV. Howles (1961) and Gooding (1975) demonstrated that heat, but not TSP, was effective in reducing seedborne viruses. Green et al. (1987) reported that treatment of tomato seed for 30 min with 12.5% TSP did not affect seed germination, but reduced the infectivity of the tobamovirus ToMV—though these researchers found this treatment to be less effective than heat. Green and Wu (1991) also reported that TSP was effective in eliminating TMV from tomato seed, without reducing seed germination. Njeru et al. (1997) reported that TSP treatment was effective in eliminating *Subterranean clover mottle virus* (SCMoV) from seed of annual clover. In order to reduce the spread of PMMoV, Lamb et al. (2001) recommended washing hands with 70% EtOH while handling plant material, and washing equipment and plant stakes with 3% TSP or house bleach. They acknowledged that seed treatments (not specified) could reduce germination. A more recent publication by the Asian Vegetable Research and Development Center (AVRDC, 2004) recommended a 10% TSP treatment for 2.5 h to control PMMoV in *Capsicum*. However, published data on the effects of a specific TSP treatment on seed viability/germination in *Capsicum* could not be found, nor could quantitative data on the effectiveness of TSP on the elimination of PMMoV from *Capsicum*.

We adopted a treatment of 10% TSP for 2.5 h, as this treatment was recommended (AVRDC, 2004) and is frequently utilized in public and private sector research programs to control PMMoV. We observed no difference in germination or percent abnormal seedlings with untreated seed or seed soaked in tap water for 2.5 h (data not shown). However, as Table 2 indicates, a 10% TSP treatment for 2.5 h significantly reduced germination percentages in 11/50 (22%) of the seed lots examined. The average reduction in germination across these lots was ~19.5%. In addition, this treatment resulted in a significantly higher percentage of abnormal seedlings in 21 (42%) of the seed lots (average increase ~175%), while reducing the percentage of abnormal seedlings in four (8%) seed lots (average reduction ~140%). The 50 seed lots included in this experiment (Table 2) ranged in age from 2 years to >40 years (mean = 14.8 yr). In addition, 18/50 seed lots were infected with PMMoV. No significant difference was observed in the percent germination or the percent abnormal seedlings when comparing PMMoV-infected to non-infected seed. Further, no correlation was observed between seed age and percent reduction in germination following TSP treatment.

Table 2. Germination of *Capsicum* seed and percentage of abnormal seedlings observed from seed soaked for 2.5 h in ddH_2O or 10% (w/v) trisodium phosphate (TSP).

		% Germination		% Abnormal seedlings	
ID#	Genus/sp.	H_2O	TSP	H_2O	TSP
297462	*C. annuum*	89	75	8	25* (p=0.011)
339058	*C. annuum*	41	41	59	65
357606†	*C. annuum*	88	90	8	20
357626	*C. annuum*	91	83	14	42* (p=0.004)
357633†	*C. annuum*	94	94	14	32* (p=0.003)
357638	*C. annuum*	77	71	14	28
368469	*C. annuum*	51	36	54	62
410407	*C. annuum*	92	80* (p=0.011)	10	46* (p=0.004)
439248†	*C. annuum*	90	65* (p=0.013)	15	24
542600	*C. annuum*	94	88	20	28
281398†	*C. baccatum*	85	82	22	44* (p=0.033)
337524	*C. baccatum*	87	92	42	52
370004†	*C. baccatum*	90	89	16	60* (p=0.028)
215699	*C. baccatum*	77	55* (p=0.015)	38	80* (p=0.001)
238061	*C. baccatum*	90	80	28	66* (p=0.005)
260567†	*C. baccatum*	94	78* (p=0.004)	14	44* (p=0.001)
159235†	*C. baccatum*	86	80	16	60* (p=0.001)
159245†	*C. baccatum*	84	75	26	45* (p=0.016)
281407	*C. baccatum*	87	81	20	48
441596	*C. baccatum*	96	80* (p=0.003)	12	44* (p=0.005)
257125†	*C. chinense*	97	88* (p=0.029)	10	28* (p=0.012)
257126	*C. chinense*	91	82	28	35
260471†	*C. chinense*	100	94	12	48* (p=0.001)
260508	*C. chinense*	98	90* (p=0.003)	22	45* (p=0.025)
281403†	*C. chinense*	95	92	24	52* (p=0.011)
360724	*C. chinense*	97	96	32	34
441608†	*C. chinense*	86	78	28	52
543208	*C. chinense*	95	97	30	48
593608	*C. chinense*	77	70* (p=0.02)	36	56
593921	*C. chinense*	68	41* (p=0.006)	51	75* (p=0.004)
159239†	*C. frutescens*	81	73	40	40
188479†	*C. frutescens*	98	95	24	45* (p=0.003)
358968	*C. frutescens*	98	96	5	10
438667	*C. frutescens*	99	93	8	24* (p=0.010)
439506†	*C. frutescens*	100	98	23	30
441642	*C. frutescens*	100	97	20	25
441653	*C. frutescens*	98	92	20	36* (p=0.005)
497984	*C. frutescens*	100	97	38	58
632917	*C. frutescens*	65	45* (p=0.001)	46	62
632930	*C. frutescens*	92	90	38	58* (p=0.012)
585262	*C. pubescens*	79	68	72	60
585266	*C. pubescens*	88	77	58	26* (p=0.001)
585267	*C. pubescens*	84	76	72	56
585268†	*C. pubescens*	86	73* (p=0.009)	52	22* (p=0.001)
585269	*C. pubescens*	93	94	70	25* (p=0.003)
585270	*C. pubescens*	89	90	50	23* (p=0.005)
585276	*C. pubescens*	91	92	39	23
585277†	*C. pubescens*	35	40	77	56
593618†	*C. pubescens*	91	90	55	40
593630	*C. pubescens*	99	93	54	38

*t-test; †PMMoV-infected

To evaluate the efficacy of TSP for eliminating PMMoV from *Capsicum* seed, we selected six accessions at random from among those shown to be infected with PMMoV via the ELISA screening. These were subjected to a 0 (control), 1 h or 2.5 h exposure to 10% TSP. As Table 3 indicates, neither treatment completely eliminated the presence of the virus, though the 2.5 h treatment resulted in a reduction in the frequency and severity of symptoms on the indicator plants, as evaluated visually.

Variability in the response of seed of different genotypes and seed lots to TSP treatment has been reported (Green et al., 1987). We suspect that the efficacy of the TSP treatment is influenced by the concentration of the virus within the seed, PMMoV strain variation, and on the location of the virus within the seed. Genda et al. (2005) demonstrated that PMMoV was generally localized in the epidermis and parenchyma of most infected seeds. It was not detected in either the endosperm or the embryo. However, in some seeds, the virus was present only on the outer surface of the epidermis and placenta. Thus, there were two distinct distribution patterns. Those authors suggested that a modification of seed treatments that enhanced the penetration of anti-viral chemical agents into the deeper tissues of the seed might increase their efficacy by enabling them to reach the virus in the deeper tissues (Genda et al., 2005).

To evaluate this hypothesis, we performed an additional indicator plant assay using extracts of seed soaked in 10% TSP for 24 h to permit greater penetration of the chemical agent. As Table 4 indicates, this treatment resulted in the complete elimination of active virus from four of the six seed lots examined, and greatly reduced, but did not eliminate it from the remaining two. This observation supports the suggestion of Genda et al. (2005) and suggests that the efficacy of TSP in eliminating PMMoV from *Capsicum* seed may be dependent on the distribution(s) of the virus within the seed and the access of the chemical agent to the virus. Seed in which only the superficial tissues are infected might respond more favorably to the TSP treatment than those in which the virus particles are present in the deeper tissues. However, what cultural or

TABLE 3. Effect of TSP on elimination of PMMoV from seed of *Capsicum* spp. treated for 0, 1 or 2.5 h. Seed extracts were used to inoculate two *Chenopodium giganteum* plants per treatment. Values represent number of inoculated plants/number of plants showing local lesion symptoms 14 d later.

	Hours of TSP treatment		
Accession ID	0	1	2.5
PI 131352	2/2	2/1	2/0
PI 271322	2/2	2/2	2/1
PI 379126	2/2	2/1	2/1
PI 407450	2/2	2/1	2/0
PI 508440	2/2	2/2	2/1
PI 585440	2/2	2/1	2/1

TABLE 4. Virus symptoms on *Chenopodium giganteum* 14 d after inoculation with buffer extracts of PMMoV-infected *Capsicum* seed treated with 10% TSP for 24 h (-T) or untreated (-C).

	No. lesions				
Accession ID	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
PI 222133-C	1	3	4	0	1
PI 222133-T	0	0	0	0	0
PI 439216-C	15	8	31	14	26
PI 439216-T	1	3	4	0	1
PI 439258-C	9	12	32	6	38
PI 439258-T	0	0	0	0	0
PI439485-C	15	42	23	43	19
PI 439485-T	5	0	0	0	5
PI 508433-C	26	30	20	24	24
PI 508433-T	0	0	0	0	0
PI 596052-C	6	6	8	12	20
PI 596052-T	0	0	0	0	0

environmental conditions foster the differential infection of the internal tissues is not known. Howles (1961) noted that older dried seed did not respond as favorably to virus elimination treatments as newly harvested seed. All seed in the present study were dried and stored for at least one year.

While longer TSP exposure or additives that enhance TSP penetration into the seed may increase its overall effectiveness, this will likely result in increased physiological damage and a greater reduction in germination. Variability in the response of individual seed lots may be attributable to the sampling method used. That is, not all seeds from individual fruit, or all fruit from an infected plant, are infected. Hence, when sampling seed for the presence of the virus, every effort should be made to utilize a truly representative sample. The observed variability in response to TSP may also be due to the differential response of PMMoV strains to inactivation. Variability in the efficacy of the control of PMMoV with TSP seed treatments may be a result of variability within or among lines for the extent of the virus in those seed tissues that serve as the predominent source of inoculum during or subsequent to germination. Gooding (1975) suggested that virus in the endosperm which was not inactivated by chemical treatment probably seldom, if ever, resulted in seedling infection. Demski (1981) noted that virus particles on the external seed coat were mainly responsible for the carryover of TMV from seed to seedling.

SUMMARY

Approximately 33% of the 4403 seed inventories in the USDA/ARS *Capsicum* germplasm collection tested positive for the presence of PMMoV. We confirmed the identification of this virus in a randomly-selected accession via amplification and sequencing of a portion of the CP gene. Treatment of PMMoV-infected

seed with 10% TSP for 2.5 h reduced or eliminated the virus in all instances while simultaneously reducing the germination and increasing the percentage of abnormal seedlings in some seed lots. Treatment of PMMoV-infected seed with TSP prior to planting is an effective means for genebanks to reduce the presence of the virus in seed of *Capsicum* spp. during the regeneration process.

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